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REMARKS

Status of the claims

Claims 1-39 were pending in the present application. Claims 1-14, 22-27, 32-34, and 39 have been withdrawn as drawn to non-elected inventions. By virtue of this response, claims 20, 21, 30, 31, 37 and 38 have been cancelled and claims 15, 17, 18, 28, and 35 have been amended, without prejudice or disclaimer of any previously-claimed subject matter. Accordingly, claims 15-19, 28-29, and 35-36 are currently under consideration.

Support for the amendments to the claims can be found throughout the disclosure and claims as originally filed. More particularly, support for claim 15 can be found for example at page 6, from line 22 to line 10 at page 7. Further support for claim 17 can be found for example at page 4, lines 2-6 and lines 15-20; at page 6, lines 13-15; as well as at page 8 from line 19 to line 9 at page 9. Further support for claim 18 can be found for example at page 6, from line 22 to line 10 at page 7. Further support for claim 15 can be found for example at page 12, between lines 13-20, and at page 13 between lines 5-14. Further support for claims 28 and 35 can be found for example at page 5, between lines 5-15, and at page 10 between lines 1-5. No new matter has been added by the foregoing amendments.

With respect to all amendments and cancelled claims, Applicants have not dedicated to the public or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional applications.

Withdrawal of previously-elected claims

Applicants note that the Examiner considers that claims 15-21, 28-31 and 35-38 are under consideration, since he alleges that claims 1-14 were "inadvertently included in Group II because of a typographical error". Initially, Group II comprised claims 1-21, 28-31 and 35-38. Applicant respectfully submits that claims 1-21, 28-31 and 35-38, as grouped initially together, satisfy the

unity of invention criteria of the PCT, the instant application being based on PCT application/CA00/00145. Further, Applicants note that U.S. Patent No. 5,789,654 contains 13 claims, 5 of which are directed to a transgenic animal and 8 of which are directed to a method of screening using the transgenic animal (of the preceding claims). Applicants respectfully disagree with the fact that "the burden required searching the method steps of Group II, with the animal, cells and method steps of Group I [would] be undue". In any event, response is given hereinbelow, on the claims considered by the Examiner to be in Group II, in accordance with the Office Action of January 16, 2004. However, Applicants respectfully request the Examiner to consider rejoinder of claims 1-14 based on the reasoning above and because of the confusion with respect to the original restriction requirement.

Sequence listing

The Office Action states that the application "fails to comply with the requirements of 37 C.F.R. 1.821 through 1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures." No Notice to Comply was attached to the copy of the Office Action received by Applicants. However, Applicants previously filed a sequence listing and a preliminary amendment to add sequence identification numbers to the specification and claims on April 2, 2002, copies of which are attached as Exhibit A, along with copies of the accompanying transmittal documents, return receipt postcard, and express mailing receipt. Sequence identifiers had also been inserted in claims 23 and 33 in the Preliminary Amendment filed August 8, 2001, a copy of which is attached as Exhibit B, along with copies of the accompanying transmittal documents, return receipt postcard, and express mailing receipt.

In view of the foregoing, Applicants respectfully submit that they are in compliance with the sequence disclosure rules.

Objection to the specification

The Office Action states that the specification is objected to for not referring to U.S. Provisional Application No. 60/119,024. By virtue of this amendment, the specification has been amended to include a section cross-referencing related applications.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the objection to the specification.

Rejection under 35 U.S.C. § 101

Claims 15-21, 28-31 and 35-38 have been rejected under 35 U.S.C. § 101, because the Examiner alleges that the claimed invention is not supported by either a specific or substantial asserted utility or a well-established utility. The Applicants respectfully traverse the rejection as follows.

More particularly, the Examiner states that the "method claimed does not have a use because the mouse described in the specification does not have a use". The applicant respectfully disagrees. The knock-out mice in accordance with one embodiment of the transgenic knock-out animals of present invention has sufficient specific and substantial utility to be in use by at least two companies. These companies use the mouse to validate the specificity of compounds suspected of acting on ERRα. In one particular embodiment, the companies have identified a compound (or pool thereof) suspected of acting on ERRα, use the ERRα knock-out mouse to ensure that the compound in fact acts through ERRα. The Examiner is absolutely correct, when he states that the knock-out ERRα mice "do not express ERRα" protein. These knock-out mice therefore have the substantial and specific utility of providing, among other utilities, a powerful control to ensure that a compound suspected of modulating weight gain, lipid metabolism, or other parameters or functions of the present invention, is effected via a targeting of ERRα. It should also be noted that the ERRα mouse exemplified in the present invention, retains its promoter and sufficient mRNA sequences, to enable a tracking of ERRα mRNA level. Consequently, this exemplified knock-out ERRα mouse also finds substantial and specific utility in that it can serve to screen for compounds that modulate the

level of ERRα expression at the transcription level, an important mechanism in ERRα expression control. Finally, the knock-out ERRα transgenic animals of the present invention can be a knock-in animal by the expression of a non-endogenous ERRα gene: whether wild-type, mutant, from a related species or from a more distant species (see for example, the disclosure at page 4, lines 2-6).

The Examiner alleges that the so-called knock-in animals ("animals having a disruption of ERRα and an insertion of a non-endogenous ERRα gene") are not taught to "have a normal phenotype or that such a mice provide any use over wild-type animals". The Applicant respectfully submits that the power of genetics, the knowledge of the genetic make-up of the transgenic animal (such as a mouse), the knowledge of the expression level of ERRα, when used in a screening assay or otherwise, provides a significant advantage. The Applicant respectfully submits, that the utility of a knock-in animal in accordance with the present invention, is not negated by the fact that under certain conditions a "wild-type animal" could be used. Mutants of ERRα can be screened to acknowledge the specific action of a compound on ERRα, agonistic mutations of ERRα can also be tested to assess the specific action of a compound on ERRα.

In view of the identification of ERRα as a key target in lipogenesis, fatty acid esterification, fatty acid oxidation, weight modulation, etc., Applicants respectfully submit that it should be clear that the transgenic animals of the present invention whether knock-out or knock-in indeed have substantial and specific utility.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 101.

Rejection under 35 U.S.C. § 112 first paragraph

The Examiner has rejected claims 15-21, 28-31 and 35-38 under 35 U.S.C. § 112, first paragraph as allegedly non-enabled. The Examiner states that "since the claimed invention is not supported by either a specific or substantial utility or a well established utility...one skilled in the art would not know how to use mice having a disruption in the ERRα gene as claimed".

Applicants respectfully traverse this rejection and submit that having shown above that the transgenic knock-out animals of the present invention do have specific and substantial utility it is respectfully submitted that this rejection has been overcome.

The Examiner alleges that "The state of the art at the time of filing [February 8, 2000] was such that embryonic stem (ES) cell technology had only been successful in mice". The Applicant notes that the Examiner bases his arguments on references published in 1995 and 1996, several years prior to the filing date of the present application. In any event, the Applicant respectfully disagrees with that statement of the Examiner and respectfully submits that at the time of filing the application the production of a transgenic animal using ES cells was known in the art.

The Examiner also alleges that "The specification does not teach the nucleic acid sequence of the ERRα gene in non-mice, non-human species or correlate the ERRα gene in mice to the ERRα gene in other species. The Examiner is referred to the Disclosure at page 37, lines 4-9:

> "The present invention therefore strongly indicates that ERRα is a direct regulator of fundamental cellular function. It is thus expected that this cellular function should occur across species. The presence of the ERRα gene and its conservation among species (**human, mice, rats, fish and lower organisms**; Escriva et al. (1997) Proc. Natl. Acad. Sci. USA 94:6803-6808), support its essential role in physiology. " [emphasis added]

In any event, without acquiescing to the rejection and solely to expedite prosecution of this application, the claims have been amended to relate to mice transgenic animals which can in the case of knock-ins express mouse or human ERRα. Support in the disclosure for "non-endogenous transgene" can be found at page 23, lines 19-23. The subject matter of withdrawn claim 8, when inserted in the method claims (if it cannot be rejoined in Group II) will reflect the fact that "said non-endogenous ERRα is a human ERRα gene or mouse ERRα gene". Applicants reserve the right to prosecute other ERRα sequences or other species of transgenic animals in further applications.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 15-21, 28-31 and 35-38 have been rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite. The Applicants respectfully traverse the rejections as follows.

The rejection of claim 15 as being dependent on claim 5 (not expressing ERRα) has been rendered moot by making it dependent on the transgenic mouse of claim 8, having a non-endogenous ERRα gene from human or mouse. The Examiner also alleges that "the method to 'determine' whether a compound increases or decreases ERRα activity in step b) cannot be determined". The Applicant respectfully submits that in view of the teachings in the disclosure that ERRα binds to hormone response elements (page 2, lines 4 to 7); that it interacts with Erα (page 2 lines 12-15); that it is involved in numerous physiological processes (page 2, lines 9-12); that it interacts with ERRβ and/or ERRγ (page 5 line 8); and that it affects many physiological and metabolic parameters or functions, a person of ordinary skill would consider that numerous means of determining the modulation of ERRα activity are possible.

The Examiner rejects claim 16 alleging that "it is unclear how to use such parameters to determine" the modulating effect of a compound on ERRα. The Applicant respectfully submits that in view of the discussion above with respect to claim 15, and in view of the teachings of the present invention as to the parameters which are affected by the knock-out of ERRα, it should be clear to the person of ordinary skill, that some parameters (*e.g.*, lipogenesis) indeed will correlate with increase or decrease of ERRα activity and/or level (page 45, lines 6-15), while the body temperature could on the other hand not be affected, thereby supporting a specific effect on ERRα.

With respect to the alleged indefiniteness of "synthetic hepatic function" in claim 16, the Examiner is referred to page 41, lines 1-3 and Table 2, which define this terminology.

The rejection of claim 17 for the use of "compounds suspected of being a modulator of ERRα," is submitted to have been overcome by the amendment which has been made solely to

clarify this claim. It should be noted that claim 17 now is dependent on the knock-out mouse of claim 4.

The Examiner rejects claim 18, for the lack of endogenous ERRα in "claim 5". In view of the dependency of claim 18 on claim 8, this objection is submitted to have been overcome. The Examiner's objections to the terminologies "promoter...being modulated by ERRα", and "agents suspected of modulating..." have been rendered moot by the deletion thereof. With respect to the allegation that the specification "does not define such promoters or provide an assay for determining such promoters. Nor were such promoters taught in the art at the time of filing". The Applicant refers the Examiner to SEQ ID NOs 1 and 2, describing cis-acting elements on which ERRα binds; to Sladek et al. 1997 MCB 17, 5400-5409 (on which two of the 3 co-authors are inventors of the present invention), and referenced at page 5, line 7, and entitled "The Orphan Receptor Estrogen-Related Receptor α Is a Transcriptional Regulator of the Human Medium-Chain Acyl Coenzyme A Dehydrogenase Gene); to page 16, at line 23 to page 17, line 6, etc. Clearly, the disclosure teaches promoters on which ERRα acts, and they were known in the art at the time of filing.

The Examiner rejects claim 28 for the use of "or related factors". This terminology has been replaced by "ERRβ or ERRγ", in accordance with the teachings at page 31, lines 1-4 "It should be noted that U.S.P. 5,298,429 also teaches the sequence of ERRα and ERRβ from human, as well as the significant conservation in the sequence of ERRα, ERRβ, and related family members." Further the Examiner's objection to the terminology in step a) is submitted to have been overcome by the instant amendment. The antecedent problem in step b) has also been corrected. The Examiner's objection relating to the "alternative condition" as well as the "first measuring" have been corrected. The terminology "suspected of modulating" has been deleted. The second "measuring" terminology has also been clarified as requested by the Examiner.

The Examiner's objection relating to claims 30 and 31 have been rendered moot by the cancellation of these claims.

With respect to claim 35, the Examiner reiterates the same objections as for claim 28. Since the amendments to claim 35 are identical to those for claim 28, it is respectfully submitted that all the objections to claim 35 have been overcome.

In view of the above and foregoing, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 514012000200. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: July 16, 2004

Respectfully submitted,

By Jill A. Jacobson

Jill A. Jacobson
Registration No.: 40,030
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304
(650) 813-5876

ATTORNEY DOCKET: 514012000200 DATE: April 2, 2002
SERIAL NO. 09/925,720 FILING DATE: August 8, 2001
INVENTOR(S): Vincent GIGUERE et al
TITLE: NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRalpha

Papers enclosed:

Transmittal Form
Fee Transmittal - in duplicate
Supplemental Preliminary Amendment (6 pgs)
Petition for Extension of Time
Prosecution by Assignee and Power of Attorney (2 pgs)
Copy of Notice to File Missing Parts of Nonprovisional Application (2 pgs)
Response to comply with requirements for patent applications ...(2 pgs)
Paper copy of Sequence Listing
CRF of Sequence Listing
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SERIAL NO. 09/925,720 FILING DATE: August 8, 2001
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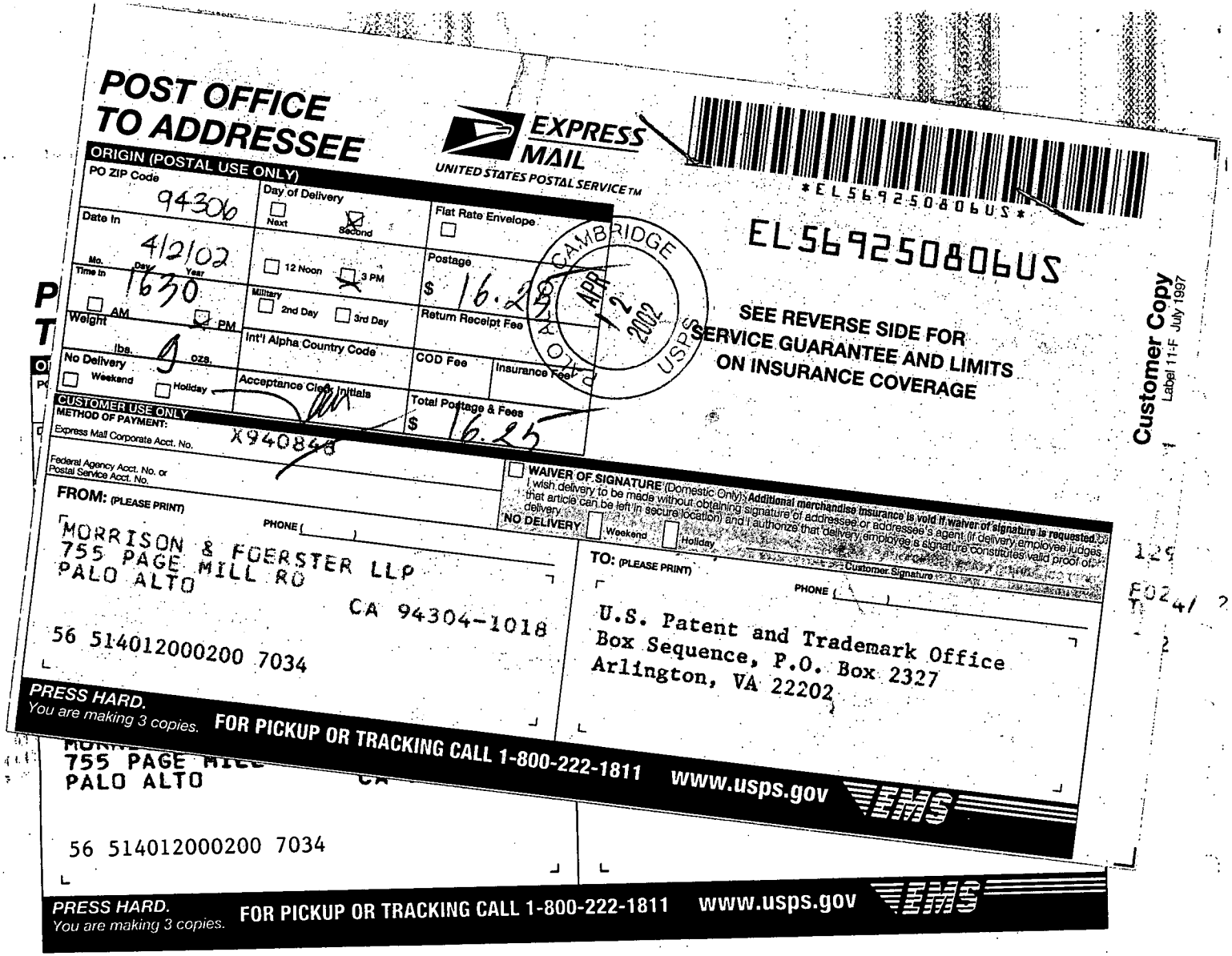

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TRANSMITTAL FORM

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Total Number Of Pages In This Submission	28

Application Number	09/925,720
Filing Date	August 8, 2001
First Named Inventor	Vincent GIGUERE
Group Art Unit	1645
Examiner Name	To Be Assigned
Attorney Docket No.	514012000200

ENCLOSURES (check all that apply)

- [X] **Fee Transmittal Form- in duplicate**
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- [X] **Supplemental Preliminary Amendment (6 pgs)** / Reply
- [ ] After Final
- [ ] Affidavits/declarations
- [X] **Extension of Time Request**
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [X] **Response to Missing Parts/ Incomplete Application**
  - [X] **Response to Missing Parts under 37 CFR 1.52 or 1.53**
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- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
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****Paper copy of Sequence Listing**
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****Statement under 37 CFR 3.73(b) with *copy* of Assignment attached (5 pgs total)**
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SIGNATURE OF APPLICANT, ATTORNEY OR AGENT

Firm or Individual Name	Gladys H. Monroy (32, 430) Morrison & Foerster LLP 755 Page Mill Road Palo Alto, CA 94304
Signature	Gladys Monroy
Date	April 2, 2002

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pa-681371

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Complete if Known	
Application Number	09/925,720
Filing Date	August 8, 2001
First Named Inventor	Vincent GIGUERE
Examiner Name	To Be Assigned
Group Art Unit	1645
Attorney Docket No.	514012000200

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☒ Applicant claims small entity status. See 37 CFR 1.27

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107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	

SUBTOTAL (1) ($)370

2. EXTRA CLAIM FEES

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Independent Claims	5-3**=	2	x	42	=	$84
Multiple Dependent					=	$0

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claims, if not paid
109	84	209	42	**Reissue independent claims over original patent
110	18	210	9	**Reissue claims in excess of 20 and over original patent

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127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	720
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions of the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per properties (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
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SUBMITTED BY

Complete *(if applicable)*

Name (Print/Type)	Gladys H. Monroy	*Registration No. (Attorney/Agent)*	*32,430*	*Telephone*	650-813-5711
Signature	Gladys Monroy			*Date*	April 2, 2002

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Total Claims	39-20**=	19	x	9	=	$171
Independent Claims	5-3**=	2	x	42	=	$84
Multiple Dependent					=	$0

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claims, if not paid
109	84	209	42	**Reissue independent claims over original patent
110	18	210	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)255

** or number previously paid, if greater; For reissues, see above.

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Fee Code	Entity Fee ($)	Small Fee Code	Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	65
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	720
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions of the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per properties (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)785

SUBMITTED BY

Complete *(if applicable)*

Name (Print/Type)	Gladys H. Monroy	Registration No. (Attorney/Agent)	32,430	Telephone	650-813-5711
Signature	Gladys Monroy			Date	April 2, 2002

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

PATENT
Docket No. 514012000200
Client Reference 760/12326.50

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Express Mail Label No.: EL569250806US Date of Deposit: April 2, 2002

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Tamara Alcaraz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Vincent GIGUERE et al.

Application No.: 09/925,720

Filing Date: August 8, 2001

For: NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRalpha

Examiner: To Be Assigned

Group Art Unit: 1645

SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-captioned application, please enter the following amendments and remarks.

AMENDMENTS

In the Specification:

Please substitute the following for the paragraph beginning on page 13, line 10

ii) TGA AGG TCA (SEQ ID NO:2);

Please substitute the following for the paragraph beginning on page 14, line 17

ii) TGA AGG TCA (SEQ ID NO:2);

Please substitute the following for the paragraph beginning on page 16, line 23 and ending on page 17, line 6

The terminology "estrogen response elements" or "estrogen cis-acting elements" refers to well-known nucleic acid sequences to which transcription factors such as the orphan nuclear receptor ERRα can bind, thereby having the potential to modulate the promoter activity of a promoter comprising such response or cis-acting elements. These cis-acting elements or estrogen response elements also termed "ERE" or "IR3" are well-known in the art (Petterson, 1996, Mech. Dev. 54:211-223). In Petterson et al. (1996, *supra*), it is for example taught that the perfect inverted repeat (IR) of the estrogen response element to which ERRα can bind has sequence AGG TCA NNN TGA CCT (SEQ ID NO:1). It is also known from Sladek et al., 1997, Bonnelye et al., 1997 and Johnston et al., 1997 that this acting element comprising the sequence TGA AGG TCA (SEQ ID NO:2) can also bind ERRα and related factors.

In the Claims:

Please amend the following claims.

23. (Amended) A method of modulating fat tissue growth and/or weight gain, comprising:

a) administering to an animal an agent which modulates the promoter activity of a gene, wherein said promoter comprises cis-acting elements selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA (SEQ ID NO:2);

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii

such as to modulate the level of said gene, thereby modulating fat tissue growth and/or weight gain in said animal.

33. (Amended) A method of treating and/or preventing obesity, comprising administering to an obese animal, or an animal susceptible of becoming obese, an agent which modulates the promoter activity of a promoter comprising a cis-acting element selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA (SEQ ID NO:2);

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii

wherein cis-acting element is capable of binding to ERRα.

REMARKS

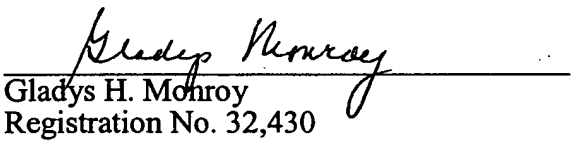
The above amendments to the specification and claims have been made to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequences. Accordingly, Applicants believe no new matter is added by these amendments.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

In the unlikely event that the Fee Transmittal becomes separated or the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 514012000200. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: April 2, 2002

By: 

Gladys H. Monroy
Registration No. 32,430

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5711
Facsimile: (650) 494-0792

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please substitute the following for the paragraph beginning on page 13, line 10

ii) TGA AGG TCA (SEQ ID NO:2);

Please substitute the following for the paragraph beginning on page 14, line 17

ii) TGA AGG TCA (SEQ ID NO:2);

Please substitute the following for the paragraph beginning on page 16, line 23 and ending on page 17, line 6

The terminology "estrogen response elements" or "estrogen cis-acting elements" refers to well-known nucleic acid sequences to which transcription factors such as the orphan nuclear receptor ERRα can bind, thereby having the potential to modulate the promoter activity of a promoter comprising such response or cis-acting elements. These cis-acting elements or estrogen response elements also termed "ERE" or "IR3" are well-known in the art (Petterson, 1996, Mech. Dev. 54:211-223). In Petterson et al. (1996, *supra*), it is for example taught that the perfect inverted repeat (IR) of the estrogen response element to which ERRα can bind has sequence AGG TCA NNN TGA CCT (SEQ ID NO:1). It is also known from Sladek et al., 1997, Bonnelye et al., 1997 and Johnston et al., 1997 that this acting element comprising the sequence TGA AGG TCA (SEQ ID NO:2) can also bind ERRα and related factors.

In the Claims:

Please amend the following claims.

23. (Twice Amended) A method of modulating fat tissue growth and/or weight gain, comprising:

a) administering to an animal an agent which modulates the promoter activity of a gene, wherein said promoter comprises cis-acting elements selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA (SEQ ID NO:2);

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii[)]

such as to modulate the level of said gene, thereby modulating fat tissue growth and/or weight gain in said animal.

33. (Twice Amended) A method of treating and/or preventing obesity, comprising administering to an obese animal, or an animal susceptible of becoming obese, an agent which modulates the promoter activity of a promoter comprising a cis-acting element selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA (SEQ ID NO:2);

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii[)]

wherein cis-acting element is capable of binding to ERRα.

PATENT
Docket No. 514012000200

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Tamara Alcaraz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Vincent GIGUERE et al.

Application No.: 09/925,720

Filing Date: August 8, 2001

For: NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRALPHA

Examiner: To Be Assigned

Group Art Unit: 1645

RESPONSE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

U.S. Patent and Trademark Office
Box Sequence, P.O. Box 2327
Arlington, VA 22202

Dear Sir:

This communication is being submitted in order to comply with requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures received within the Notice to File Missing Parts of Nonprovisional Application dated October 2, 2001.

REMARKS

The printed Sequence Listing is identical to the Sequence Listing submitted in machine-readable form.

This paper is submitted to comply with the United States Patent Office rules governing gene sequences. No new matter is added by the submission of this sequence listing.

In the unlikely event that the fee transmittal is separated from this sequence listing and the U.S. Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this sequence listing to our **Deposit Account No. 03-1952**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: April 2, 2002

By: Gladys Monroy

Gladys H. Monroy
Registration No. 32,430

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5711
Facsimile: (650) 494-0792

SEQUENCE LISTING

<110> Giguere, Vincent
Sladek, Robert
Luo, Jiang-Ming

<120> NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRALPHA

<130> 514012000200

<140> US 09/925,720
<141> 2001-08-08

<150> US 60/119,024
<151> 1999-02-08

<150> PCT/CA00/00145
<151> 2000-02-08

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<212> DNA
<213> Mus musculus

<400> 2
tgaaggtca 9

Morrison & Foerster llp

755 Page Mill Road
Palo Alto, California 94304-1018
(650) 813-5600

FORMAT: ASCII
APPLICANT: Giguere, Vincent et al.
TITLE: NON-HUMAN TRANSGENIC
ANIMAL WHOSE GERM CELLS
AND SOMATIC CELLS CONTAIN KNOCKOUT
MUTATION IN DNA ENCODING ORPHAN NUCLEAR
RECEPTOR ERRALPHA
DOCKET NO: 514012000200
SERIAL NO.: 09/925,720
DATE RECORDED: April 2, 2002
COMPUTER: IBM PC COMPATIBLE
OPERATING SYSTEM: PC-DOS/MS-DOS

ATTORNEY DOCKET: 514012000200
INVENTOR(S): Vincent GIGUERE et al.

DATE: August 8, 2001

TITLE: NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRalpha

Papers enclosed:

- ☒ TRANSMITTAL UNDER RULE 1.53(c)
- ☒ SPECIFICATION (INCLUDING CLAIMS) (58 PAGES)
- ☒ PRELIMINARY AMENDMENT (32 SHEETS)
- ☒ DRAWINGS (11 SHEETS)
- ☒ APPLICATION DATA SHEET (2 PAGES)
- ☒ OTHER: Fee Determination Record and Return receipt postcard

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- ☒ SPECIFICATION (INCLUDING CLAIMS)...
- ☒ PRELIMINARY AMENDMENT (32 SHEETS)
- ☒ DRAWINGS (11 SHEETS)
- ☒ APPLICATION DATA SHEET (2 PAGES)
- ☒ OTHER: Fee Determination Record and Return receipt postcard

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	514012000200
First Inventor	Vincent GIGUERE
Title	NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRalpha
Express Mail Label No.	EL569250718US

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Express Mail Label No.: EL569250718US Date of Deposit: August 8, 2001

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Tamara Venegas

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ Fee Transmittal Form (e.g. PTO/SB/17) *(Submit an original, and a duplicate for fee processing)*
2. ☒ Applicant claims small entity status. See 37 CFR 1.27.
3. ☒ Specification [*Total Pages* 49]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. ☒ Drawing(s) *(35 USC 113)* [*Total Sheets* 11]
5. Oath or Declaration [*Total Pages* ☐]
   a. ☐ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b)
6. ☒ Application Data Sheet. See 37 CFR 1.76

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ paper
   c. ☐ Statements verifying identify of above copies

ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement *(where there is an assignee)* ☐ Power of Attorney
11. ☐ English Translation document (if applicable)
12. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☒ Preliminary Amendment-32 pages
14. ☒ Return Receipt Postcard (MPEP 503) *Should be specifically itemized)*
15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☐ Request and Certification under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☒ Other Fee Determination Record

18. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No:

Prior application information: Examiner Group / Art Unit:

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. This incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS

☒ *Customer Number or Bar Code Label*

25226
PATENT TRADEMARK OFFICE

and/or ☒ *Correspondence address below*

Name	Morrison & Foerster, LLP				
Address	755 Page Mill Road				
City	Palo Alto	*State*	CA	*Zip Code*	94304
Country	USA	*Telephone*	650-813-5711	*Fax*	650-494-0792

Name (Print/Type)	Thomas E. Ciotti for Gladys H. Monroy	*Registration No. (Attorney/Agent)*	Ciotti - 21,013; Monroy - 32,430
Signature		*Date* August 8, 2001	

PATENT
Docket No. 514012000200
Client Reference 760/12326.50

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Tamara Venegas

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Vincent GIGUERE et al.

Serial No.: To Be Assigned

Filing Date: Herewith

For: NON-HUMAN TRANSGENIC ANIMAL WHOSE GERM CELLS AND SOMATIC CELLS CONTAIN A KNOCKOUT MUTATION IN DNA ENCODING ORPHAN NUCLEAR RECEPTOR ERRalpha

Examiner: To Be Assigned

Group Art Unit: To Be Assigned

PRELIMINARY AMENDMENT

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination of the above-captioned application, please enter the following amendments and remarks.

AMENDMENTS

In the Specification:

Please substitute the following for the paragraph beginning on page 1, line 7 and ending on line 24

The present invention relates to a transgenic non-human animal whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα. More particularly, the invention relates to a non-human transgenic mammal whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα and more specifically to a transgenic mice whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα. In one particular embodiment, mice containing a disruption of both copies of the ERRα gene lack detectable expression of the ERRα protein. The invention further relates to such knockout non-human animals which express an Errα gene which is different from the endogenous gene which was disrupted. In a particular embodiment, the invention relates to a transgenic mouse having its endogenous ERRα gene disrupted and expressing human ERRα. As well, the invention relates to cell lines in which ERRα activity (and/or level) has been inactivated or augmented. The invention further relates to uses and methods of the transgenic animals of the present invention to select agents which modulate the expression and/or activity of ERRα and to agents identified by these methods.

Please substitute the following for the paragraph beginning on page 2, line 16 and ending on page 3, line 6

Obesity is a prevalent disorder that often leads to diabetes, cardiovascular disease, and joint disorders. Although the precise mechanism which leads to the development of obesity has yet to be precisely determined, it appears clear that a number of mechanisms, which normally function to maintain homeostasy and normal body weight are involved. Transgenic mice with an induced brown fat deficiency have indicated that this tissue is implicated in the control of the balance of in mice (Lowell et al., Nature 366:740-742, 1993). Further, a correlation between brown adipose tissue dysfunction and obesity and diabetes has been reported (Lowell et al., *Supra*). Previous studies have demonstrated that ERRα is highly expressed in brown adipose tissue (BAT)

during murine development and that the receptor is upregulated during white and brown adipocyte differentiation *in vitro* (Sladek et al., 1997; Vega and Kelly, 1997). In addition, ERRα has been shown to modulate the activity of the medium chain acyl-coA dehydrogenase (MCAD) promoter, a key regulatory step in the fatty acid β-oxidation pathway (Sladek et al., 1997; Vega and Kelly, 1997). More recently, a transgenic mouse whose germ cells and somatic cells contain a knockout mutation in DNA encoding an endogenous beta.$_3$-adrenergic receptor polypeptide, thereby obtaining a mouse having a modest increase in body fat, has been reported (U.S. 5,789,654).

Please substitute the following for the paragraph beginning on page 8, line 19 and ending on page 9, line 9

It will also be apparent that the cells and tissues of the transgenic animals of the present invention can be useful in *in vitro* methods relating to fat deposition and related disorders (including rational design and/or screening of compounds which can modulate expression and/or activity of the ERRα orphan nuclear receptor. In a related aspect, the present invention further relates to cell lines in which the activity of ERRα has been inactivated or augmented. In addition to being derived from the transgenic animals of the present invention, such cell lines, can for example be derived as commonly known in the art using the construct of the present invention or derivatives or variants thereof. Such cell lines can be used similarly to the animals of the present invention to identify compounds which modulate ERRα level and/or activity, dissect the physiological and biochemical function (including structure/function relationships, as they relate to fat deposition and the like) of ERRα. Thus, the present invention also relates to established cell lines or primary cells derived from an animal of the present invention. In one embodiment, fat pads from a transgenic mouse of the present invention was used to obtain primary cells which were grown and used in *in vitro* methods (i.e. insulin effect, glucose uptake, lipogenesis measurements and the like). Such experiments validated these cells as a pertinent tool for the methods and uses of the present invention.

Please substitute the following for the paragraph beginning on page 11, line 10 and ending on line 11

(c) transplating the animal zygote into a pseudopregnant compatible animal;

Please substitute the following for the paragraph beginning on page 13, line 11

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

Please substitute the following for the paragraph beginning on page 14, line 17

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

Please substitute the following for the paragraph beginning on page 15, line 17 and ending on page 16, line 2

As used herein, "non-human transgenic animal" is any non-human animal in which at least one cell comprises genetically altered information through known means such as microinjection, virus-delivered infection, or homologous recombination. In one particularly preferred embodiment of the present invention, the transgenic animal is a transgenic mouse, in which the genetic alteration has been introduced in a germ-line cell such, that it enables the transfer of this genetic alteration to the offsprings thereof. Such offsprings, containing this genetic alteration, are also transgenic mice.

Please substitute the following for the paragraph beginning on page 16, line 23 and ending on page 17, line 6

The terminology "estrogen response elements" or "estrogen cis-acting elements" refers to well-known nucleic acid sequences to which transcription factors such as the orphan nuclear receptor ERRα can bind, thereby having the potential to modulate the promoter activity of a promoter comprising such response or cis-acting elements. These cis-acting elements or estrogen response elements also termed "ERE" or "IR3" are well-known in the art (Petterson, 1996, Mech. Dev. 54:211-223). In Petterson et al. (1996, *supra*), it is for example taught that the perfect inverted repeat (IR) of the estrogen response element to which ERRα can bind has sequence AGG TCA NNN TGA CCT (SEQ ID NO:1). It is also known from Sladek et al., 1997, Bonnelye et al., 1997 and Johnston et al., 1997 that this acting element comprising the sequence TGA AGG TCA can also bind ERRα and related factors.

Please substitute the following for the paragraph beginning on page 22, line 3 and ending on line 20

Polymerase chain reaction (PCR) is carried out in accordance with known techniques. See, e.g., U.S. patents 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all four U.S. patents are incorporated herein by reference). In general, PCR involves, a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analysed to assess whether the sequence or sequences to be detected are present. Detection of the amplified sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophores, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al. Eds, Acad. Press, 1990).

Please substitute the following for the paragraph beginning on page 26, line 11 and ending on line 13

The functional derivatives of the present invention can be synthesized chemically or produced through recombinant DNA technology. All these methods are well known in the art.

Please substitute the following for the paragraph beginning on page 28, line 24 and ending on page 29, line 4

As well, having identified ERRα as a target for lipogenesis, fatty acid esterification and fatty acid oxydation modulation, ERRα can be used in a number of *in vitro* and *in vivo* assays to identify ligands therefor and dissect its structure/function relationship. Non limiting examples thereof include binding assays and the two hybrid system technology, as well known in the art (Ausubel et al., 1994, supra). This assay has proven beneficial to test compounds or a library thereof. Thus, the invention also covers ERRα-expressing cells (prokaryotes, lower and higher

eukaryotes) or variants thereof to identify mutations which modulate ERRα activity or compounds which have ERRα modulating effects.

Please substitute the following for the paragraph beginning on page 29, line 5 and ending on line 20

The present invention also provides antisense nucleic acid molecules which can be used for example to decrease or abrogate the expression of the nucleic acid sequences or proteins of the present invention. An antisense nucleic acid molecule according to the present invention refers to a molecule capable of forming a stable duplex or triplex with a portion of its targeted nucleic acid sequence (DNA or RNA). The use of antisense nucleic acid molecules and the design and modification of such molecules is well known in the art as described for example in WO 96/32966, WO 96/11266, WO 94/15646, WO 93/08845 and U.S. patent 5,593,974. Antisense nucleic acid molecules according to the present invention can be derived from the nucleic acid sequences and modified in accordance to well known methods. For example, some antisense molecules can be designed to be more resistant to degradation to increase their affinity to their targeted sequence, to affect their transport to chosen cell types or cell compartments, and/or to enhance their lipid solubility by using nucleotide analogs and/or substituting chosen chemical fragments thereof, as commonly known in the art.

Please substitute the following for the paragraph beginning on page 34, line 17 and ending on page 35, line 6

Figs. 1A-1D show the targeted disruption of the *Estrra* gene and heterozygote inbreeding analysis. Fig. 1A, Structure of the ERRα locus, targeting vector, and recombinant allele. Top, map of the wild type locus: exons are indicated by black boxes. E2 encodes the upstream zinc-binding motif of the ERRα DNA-binding domain. Center, targeting construct. Bottom, map of the targeted allele, showing replacement of exon 2 sequences by the *neo*^{r} cassette. The restriction enzyme digests and the probes used to characterize the knockout mice are illustrated. B, *BamH*I; H, *Hind*III. Fig. 1B, Southern blot analysis of targeted ES clones. DNA from parental ES cells (R1) and two targeted clones (57 and 62) was digested with *BamH*I and hybridized to the 3' probe. The positions of bands corresponding to the wild-type (10.7 kb) and targeted alleles (4.5 kb) are indicated (upper panel). Single integration of the targeting construct in targeted ES cell

clones was confirmed with a *neo*^{r} probe: a single hybridizing band (6.0 kb) is present in the targeted lines (lower panel). Fig. 1C, Southern blot analysis of genotypes of 28d old pups from a heterozygote intercross: the litter contains viable homozygous null mice. Fig. 1D, Northern blot analysis of RNA obtained from the kidneys of the progeny of heterozygous intercrosses. ERRα expression is not detected in RNA samples obtained from homozygous null mutants.

Please substitute the following for the paragraph beginning on page 35, line 7 and ending on line 16

Figs. 2A-2D show the phenotypic analysis of *Estrra* null mutants. Fig. 2A, Mutant animals display decreased weight gain. Growth curves were performed by weighing animals at the indicated ages: both male and female knockout mice display significantly reduced body weight in comparison to their wild-type littermates. Arrows indicate start of pre-pubertal growth spurt. Fig. 2B, Body composition of *Estrra* null mice shows decreased ratio of fat to lean mass. Fig. 2C, *Estrra*$^{-/-}$ mice contain decreased body fat. Superficial carcass dissection of two 20 week old male mice shows the decreased body fat content of a 32.9 g knockout mouse (right) in comparison with his 38.1 g wild-type littermate (left). Fig. 2D, The difference in body composition is reflected by the relative sizes of the dissected fat pads.

Please substitute the following for the paragraph beginning on page 35, line 17 and ending on line 24

Figs. 3A-3C show the analysis of intestinal lipid transport in *Estrra* null mutants. Fig. 3A, Thin layer chromatographic analysis of tissue lipid content. The intestines of Estrra$^{-/-}$ mice contain decreased triglyceride and increased free fatty acids in comparison with their wild-type and heterozygous littermates. Fig. 3B, Analysis of glycerolipid synthesis in *Estrra* null mutants. Estrra$^{-/-}$ mice demonstrate reduced triglyceride synthesis in intestinal and hepatic whole cell extracts. Fig. 3C, Fat absorption profile. Estrra$^{-/-}$ mice and littermate controls display similar rates of absorption of radiolabeled oleic acid.

Please substitute the following for the paragraph beginning on page 35, line 25 and ending on page 36, line 2

Figs. 4A and 4B show the analysis of adipocyte function in Estrra null mutants. Fig. 4A, Histologic studies of epididymal fat pads show that *Estrra*$^{-/-}$ mice (lower panel) have decreased adipocyte volume in comparison to wild-type animals (upper panel). Fig. 4B, *Estrra*$^{-/-}$ mice demonstrate decreased lipogenesis in comparison to littermate controls.

Please substitute the following for the paragraph beginning on page 36, line 13 and ending on page 37, line 3

The method of production and the transgenic animals of the present invention are described herein below. In general, these animals are produced by engineering a nucleic acid construct which can disrupt the expression of the endogenous ERRα gene (i.e., the murine ERRα gene). Using known methods, this construct is amplified in bacterial cells, purified, and transferred into ES cells or isolated oocytes. The transfected ES cells can then be injected into blastocysts to generate chimeras. The chimeras which transmit the mutation to their offspring are identified and selected. These animals can then be used as founder animals to obtain different animal lines, derived from breeding with chosen animals. Heterozygous animals can then be produced and further mated to generate a hybrid F1 cross. Further matings of the F1 heterozygotes produce the wild type, heterozygous and homozygous null mutants of ERRα (having both copies of the ERRα gene disrupted). The homozygous animals can then serve in a number of experiments. Non-limiting examples thereof include: the characterization of their phenotype, and a reconstitution of the ERRα activity by complementation by a non-endogenous copy of a wild type ERRα gene or mutant or variant ERRα gene. An animal (or cells derived therefrom) expressing a mutant form of ERRα gene (from human, for example) could be used to screen for compounds which modulate more specifically the mutant form of the ERRα gene.

Please substitute the following for the paragraph beginning on page 37, line 4 and ending on line 11

The present invention therefore strongly indicates that ERRα is a direct regulator of fundamental cellular function. It is thus expected that this cellular function should occur across species. The presence of the ERRα gene and its conservation among species (human, mice, rats,

fish and lower organisms; Escriva et al. (1997) Proc. Natl. Acad. Sci. USA 94:6803-6808), support its essential role in physiology. Thus, the antagonists identified by the methods and assays of the present invention should find a utility in the treatment of obesity and other metabolic diseases associated with ERRα malfunction in humans.

Please substitute the following for the paragraph beginning on page 37, line 12 and ending on line 13

The present invention is illustrated in further detail by the following non-limiting examples.

Please substitute the following for the paragraph beginning on page 37, line 17 and ending on page 38, line 8

Three overlapping λ clones containing the mouse *Estrra* locus were isolated from a 129Sv genomic library (gift of Dr. A. Joyner, Skirball Institute, New York) and characterized by restriction mapping and direct sequencing of the exon boundaries. The knockout construct was created using pNT (Tybulewicz et al., 1991) and contained 6.4 Kb of genomic DNA flanking the second exon of *Estrra*. An endfilled 4.2 Kb *BamHI/Not*I fragment, lying upstream of the second exon, was cloned into the *Xho*I site of pNT, while a 2.2 Kb *Hind*III fragment was cloned between the *neor* and TK cassettes to provide the 3' arm of the construct. Correct targeting of the *Estrra* locus replaces the receptor's second exon, which encodes a critical part of its DNA binding domain, with a *neo* cassette. The linearized construct was electroporated into R1 ES cells (Nagy and Rossant, 1993) which were selected with G418 (150 μg/mL) and gancyclovir (2 μM). Two ES cell clones were isolated and injected into C57BL6 blastocysts to generate chimeras, and three chimeras transmitted the mutation to their offspring. Heterozygous mice, generated by mating the chimeric animals with 129SvJ mice were mated with C57BL6 animals to generate hybrid F1 animals: physiologic studies were performed using the F2 null mutant and wild-type offspring obtained by mating the F1 hybrid heterozygotes. Complete disruption of the *Estrra* allele was verified by performing Northern blots using RNA obtained from placenta and kidneys of homozygous mutants.

Please substitute the following for the paragraph beginning on page 39, line 23 and ending on page 40, line 3

Mice were studied at 10:00h following free access to food overnight. The animals were conditioned by sham intraperitoneal injections of water. On the day of the experiment, the animals were injected intraperitoneally with ^3H_2O (0.5 mCi per 100 g body weight) and sacrificed by cervical dislocation 30 minutes later. Serum, adipose tissue and liver samples were harvested and stored at -80°C. The tissues were homogenized and heated in ethanolic KOH: the resulting extract, which contained saponified lipids, was acidified using concentrated sulfuric acid and extracted using petroleum ether. The extract was dried by evaporation and ^3H incorporation determined by scintillation counting.

Please substitute the following for the paragraph beginning on page 44, line 6 and ending on line 24

Previous studies have demonstrated that ERRα is highly expressed in brown adipose tissue (BAT) during murine development and that the receptor is upregulated during white and brown adipocyte differentiation *in vitro* (Sladek et al., 1997; Vega and Kelly, 1997). In addition, ERRα has been shown to modulate the activity of the medium chain acyl-coA dehydrogenase (MCAD) promoter, a key regulatory step in the fatty acid β-oxidation pathway (Sladek et al., 1997; Vega and Kelly, 1997). As dysregulation of BAT function has been associated with abnormalities of body composition, therefore, a characterization of BAT function in the ERRα knockout mice was carried out. ERRα null mutants had normal core body temperature and basal metabolic rate and displayed normal expression levels of uncoupling protein (UCP) mRNA in BAT (UCP-1) and skeletal muscle (UCP-2) (data not shown). Defects in fatty acid oxidation are frequently only apparent following situations of physiologic stress or food deprivation: neither prolonged cold exposure or fasts of up to 48 hour's duration resulted in any morbidity or mortality of Estrra$^{-/-}$ mice (data not shown). Taken together, these data suggest that the abnormal body composition seen in ERRα null mutants was not a result of increased thermogenesis or increased basal energy expenditure, and that the animals did not have physiologically significant defects in fatty acid β-oxidation.

Please substitute the following for the paragraph beginning on page 44, line 25 and ending on page 45, line 15

Fat pads obtained from *Estrra* mutants displayed decreased adipocyte volume in comparison to wild-type animals (Fig. 4A), which suggests that the decreased adipose tissue mass observed in Estrra$^{-/-}$ mice results from an imbalance between fatty acid synthesis and lipolysis rather than defects in adipocyte proliferation and differentiation. As ERRα expression is induced during early adipocyte differentiation *in vitro* (Sladek et al., 1997), it is possible that ERRα acts as a regulator of processes important for adipocyte function, such as fatty acid synthesis or esterification. In animals fed a standard laboratory diet, murine adipose tissue contains triglyceride formed from fatty acids that are synthesized *de novo* rather than from dietary lipid. Lipogenesis was assessed by treating *Estrra*$^{-/-}$ mice with $^{3}H_2O$: the amount of radioactive label incorporated into triacylglycerol can be measured by saponification and ether extraction of adipose tissues and other organs. *Estrra* null mutants demonstrate significantly decreased lipogenesis in comparison to littermate controls: in particular, knockout animals show a 30-55% decrease in ^{3}H incorporation into adipose tissue lipids and a 50% decrease in 3H incorporation into hepatic lipids (Fig. 4B). This observation demonstrates that adipose tissue of knockout mice possesses a defect in TG synthesis, which may result from decreased adipocyte and hepatic glycolysis activity, fatty acid synthesis or esterification.

Please substitute the following for the paragraph beginning on page 45, line 16 and ending on page 46, line 12

Experiments performed using the Estrra$^{-/-}$ mice revealed that ERRα is a key regulator of fat metabolism, including intestinal fat transfer and esterification, as well as hepatic and adipocyte fat deposition. *Estrra*$^{-/-}$ mice display decreased fat content associated with reduced intestinal fatty acid esterification rates and abnormal regulation of fat deposition and mobilization in adipocytes and liver. Previous *in vitro* studies have demonstrated that ERRα modulates the expression of MCAD, a key regulatory enzyme of fatty acid β-oxidation, a pathway which may also play a role in establishing the ERRα phenotype. The relative importance of each of these effects in establishing the body composition of ERRα mice remains to be determined. Since the *Estrra*$^{-/-}$ mice show a normal level of energy intake, one would expect to observe an increase in energy expenditure to account for the decreased fat content of these mice. However, the sensitivity

of fecal fat measurements and calorimetry experiments may not be sufficient to identify small differences between wild-type and knockout animals which over a period of time would be sufficient to explain the observed phenotype. Within these experimental limitations, the data presented herein demonstrate that ERRα mice are lean as a result of aberrant regulation of peripheral lipid mobilization. ERRα mice display an unique combination of properties that suggests that modulation of ERRα activity may provide an effective method to regulate fat metabolism and that ERRα would be a key drug target for the treatment of obesity and other disorders of fat deposition. In addition, the close linkage of ESTRRA and diabetes susceptibility locus IDDM4 (Sladek et al., 1997) together with physiological defects observed in *Estrra*$^{-/-}$ mice suggests that drugs influencing ERRα activity could also be used to treat diabetes and other metabolic disorders.

In the Claims:

Please amend the following claims.

3. (Amended) The non-human transgenic animal of claim 1, wherein said animal is a mammal.

5. (Amended) The non-human transgenic animal of claim 1, displaying a lean phenotype.

6. (Amended) The non-human transgenic animal of claim 1, whose germ cells and somatic cells additionally comprise a transgene encoding a non endogenous ERRα orphan nuclear receptor gene, wherein said transgene is expressed at levels sufficient to complement the disrupted endogenous ERRα orphan nuclear receptor activity.

9. (Amended) A cell line derived from the non-human transgenic animal of claim 1.

15. (Amended) A method for screening and identifying a compound which modulates ERRα orphan nuclear receptor activity, the method including:

a) exposing the non-human transgenic animal of claim 5 to a candidate compound, and;

b) determining the activity of said ERRα orphan nuclear receptor in said animal, wherein an increase in the receptor activity as compared to an unexposed non-human animal is indicative of a compound being capable of increasing ERRα orphan nuclear receptor activity, while a decrease in said receptor activity as compared to an unexposed non-human animal, is indicative of a compound being capable of decreasing ERRα orphan nuclear receptor activity.

18. Method of identifying an agent which modulates fat and/or glucose metabolism *in vivo* comprising:

a) providing a promoter operably linked to a selectable or assayable marker, said promoter being modulated by ERRα;

b) measuring or selecting for said marker in a presence and in an absence of an agent suspected of modulating the promoter modulating activity of ERRα, thereby identifying an agent which modulates ERRα activity wherein a difference in the transcriptional activity in the presence of said agent, as compared to that in the absence thereof, identifies said agent as a modulator of ERRα activity;

c) administering said agent identified in b) to a non-human transgenic animal according to claim 1; and

d) measuring lipid and/or glucose levels in said animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in lipid and/or glucose levels of the animal of step c) as compared to that of said control animal identifies said agent as a modulator of fat and/or glucose metabolism *in vivo*.

21. (Amended) The method of claim 20, wherein said mammal is a mouse.

22. (Amended) A modulator of fat and/or glucose metabolism *in vivo* identified by the method of claim 18.

23. (Amended) A method of modulating fat tissue growth and/or weight gain, comprising:

a) administering to an animal an agent which modulates the promoter activity of a gene, wherein said promoter comprises cis-acting elements selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA;

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii)

such as to modulate the level of said gene, thereby modulating fat tissue growth and/or weight gain in said animal.

28. (Amended) A method of determining whether an agent modulates fat tissue growth and/or weight gain in an animal comprising:

a) providing a transcriptionally active preparation of ERRα or related factors and a DNA sequence comprising a promoter having a cis-acting sequence which modulates activity thereof by an interaction thereto of said ERRα and related factors;

b) measuring said transcriptional activity of said promoter or of a binding of at least ERRα or related factors to said cis-acting sequence in a presence and in an absence of an agent suspected of modulating the transcriptional activity of said promoter or the binding of said factors to said cis-acting sequence, thereby identifying an agent which modulates transcription of said promoter and wherein a difference in the transcriptional activity and/or binding in the presence of said agent, as compared to that in the absence thereof identifies said agent as a modulator of transcription;

c) administering said agent identified in b) to a non-human transgenic animal according to claim 1; and

d) measuring fat tissue growth and/or weight gain in the animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in fat tissue growth and/or weight gain of the animal of step c) as compared to that of the control animal identifies said agent as a modulator of fat tissue growth and/or weight gain *in vivo*.

32. (Amended) A modulator of fat and/or glucose metabolism *in vivo* identified by the method of claim 28.

33. (Amended) A method of treating and/or preventing obesity, comprising administering to an obese animal, or an animal susceptible of becoming obese, an agent which modulates the promoter activity of a promoter comprising a cis-acting element selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA;

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii)

wherein cis-acting element is capable of binding to ERRα.

35. (Amended) A method of determining whether an agent modulates obesity in an animal comprising:

a) providing a transcriptionally active preparation of ERRα or related factors and a DNA sequence comprising a promoter having a cis-acting sequence which modulates activity thereof by an interaction thereto of said ERRα and related factors;

b) measuring said transcriptional activity of said promoter or of a binding of at least ERRα or related factors to said cis-acting sequence in a presence and in an absence of an agent suspected of modulating the transcriptional activity of said promoter or the binding of said factors to said cis-acting sequence, thereby identifying an agent which modulates transcription of said promoter and wherein a difference in the transcriptional activity and/or binding in the presence of said agent, as compared to that in the absence thereof identifies said agent as a modulator of transcription;

c) administering said agent identified in b) to a non-human transgenic animal according to claim 1; and

d) assessing obesity in the animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in obesity of the

animal of step c) as compared to that of the control animal identifies said agent as a modulator of obesity *in vivo*.

38. (Amended) The method of claim 37, wherein said mammal is a mouse.

39. (Amended) A modulator of glucose or fat metabolism *in vivo* identified by the method of claim 35.

REMARKS

The above amendments to the specification have been made to correct typographical and grammatical errors. Accordingly, Applicants believe no new matter is added by these amendments.

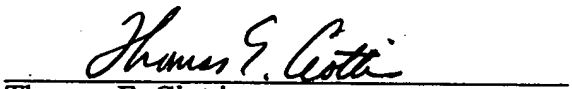
The amendments to the claims have been made to remove multiple dependency and reduce filing fees. These amendments are not intended to abandon, disclaim or dedicate any subject matter.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

In the unlikely event that the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 514012000200. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: August 8, 2001

By: 

Thomas E. Ciotti
Registration No. 21,013
for
Gladys H. Monroy
Registration No. 32,430

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5711
Facsimile: (650) 494-0792

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please substitute the following for the paragraph beginning on page 1, line 7 and ending on line 24

The present invention relates to a transgenic non-human animal whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα. More particularly, the invention relates to a non-human transgenic mammal whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα and more specifically to a transgenic mice whose germ cells and somatic cells contain a knockout mutation in DNA encoding orphan nuclear receptor ERRα. In one particular embodiment, mice containing a disruption of both copies of the ERRα gene lack detectable expression of the ERRα protein. The invention further relates to such knockout non-human animals which express an [Err] ERRα gene which is different from the endogenous gene which was disrupted. In a particular embodiment, the invention relates to a transgenic mouse having its endogenous ERRα gene disrupted and expressing human ERRα. As well, the invention relates to cell lines in which ERRα activity (and/or level) has been inactivated or augmented. The invention further relates to uses and methods of the transgenic animals of the present invention to select agents which modulate the expression and/or activity of ERRα and to agents identified by these methods.

Please substitute the following for the paragraph beginning on page 2, line 16 and ending on page 3, line 6

Obesity is a prevalent disorder that often leads to diabetes, cardiovascular disease, and joint disorders. Although the precise mechanism which leads to the development of obesity has yet to be precisely determined, it appears clear that a number of mechanisms, which normally function to maintain homeostasy and normal body weight are involved. Transgenic mice with an induced brown fat deficiency have indicated that this tissue is implicated in the control of the balance of in mice (Lowell et al., Nature 366:740-742, 1993). Further, a correlation between brown adipose tissue dysfunction and obesity and diabetes has been reported (Lowell et al., *Supra*). Previous studies have demonstrated that ERRα is highly expressed in brown adipose tissue (BAT) during murine development and that the receptor is upregulated during white and brown

adipocyte differentiation *in vitro* (Sladek et al., 1997; Vega and Kelly, 1997). In addition, ERRα has been shown to modulate the activity of the medium chain acyl-coA dehydrogenase (MCAD) promoter, a key regulatory step in the fatty acid β-oxidation pathway (Sladek et al., 1997; Vega and Kelly, 1997). More recently, a transgenic mouse whose germ cells and somatic cells contain a knockout mutation in DNA encoding an endogenous [.]beta.$_3$-adrenergic receptor polypeptide, thereby obtaining a mouse having a modest increase in body fat, has been reported (U.S. 5,789,654).

Please substitute the following for the paragraph beginning on page 8, line 19 and ending on page 9, line 9

It will also be apparent that the cells and tissues of the transgenic animals of the present invention can be useful in *in vitro* methods relating to fat deposition and related disorders (including rational design and/or screening of compounds which can modulate expression and/or activity of the ERRα orphan nuclear receptor. In a related aspect, the present invention further relates to cell lines in which the activity of ERRα has been inactivated or augmented. In addition to being derived from the transgenic animals of the present invention, such cell lines, can for example be derived as commonly known in the art using the construct of the present invention or derivatives or variants thereof. Such cell lines can be used similarly to the animals of the present invention to identify compounds which modulate ERRα level and/or [acivity] activity, dissect the physiological and biochemical function (including structure/function relationships, as they relate to fat deposition and the like) of ERRα. Thus, the present invention also relates to established cell lines or primary cells derived from an animal of the present invention. In one embodiment, fat pads from a transgenic mouse of the present invention was used to obtain primary cells which were grown and used in *in vitro* methods (i.e. insulin effect, [gucose] glucose uptake, lipogenesis measurements and the like). Such experiments validated these cells as a pertinent tool for the methods and uses of the present invention.

Please substitute the following for the paragraph beginning on page 11, line 10 and ending on line 11

(c) transplating the animal zygote into a pseudopregnant compatible animal;

Please substitute the following for the paragraph beginning on page 13, line 11

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

Please substitute the following for the paragraph beginning on page 14, line 17

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

Please substitute the following for the paragraph beginning on page 15, line 17 and ending on page 16, line 2

As used herein, "[hon]non-human transgenic animal" is any non-human animal in which at least one cell comprises genetically altered information through known means such as microinjection, virus-delivered infection, or homologous recombination. In one particularly preferred embodiment of the present invention, the transgenic animal is a transgenic mouse, in which the genetic alteration has been introduced in a germ-line cell such, that it enables the transfer of this genetic alteration to the offsprings thereof. Such offsprings, containing this genetic alteration, are also transgenic mice.

Please substitute the following for the paragraph beginning on page 16, line 23 and ending on page 17, line 6

The terminology "estrogen response elements" or "estrogen cis-acting elements" refers to well-known nucleic acid sequences to which transcription factors such as the orphan nuclear receptor ERRα can bind, thereby having the potential to modulate the promoter activity of a promoter comprising such response or cis-acting elements. These cis-acting elements or estrogen response elements also termed "ERE" or "IR3" are well-known in the art (Petterson, 1996, Mech. Dev. 54:211-223). In Petterson et al. (1996, *supra*), it is for example taught that the perfect inverted repeat (IR) of the estrogen response element to which ERRα can bind has sequence AGG TCA NNN TGA CCT (SEQ ID NO:1). It is also known from Sladek et al., 1997, Bonnelye et al., 1997 and Johnston et al., 1997 that this acting element comprising the sequence TGA AGG TCA can also bind ERRα and related factors.

Please substitute the following for the paragraph beginning on page 22, line 3 and ending on line 20

Polymerase chain reaction (PCR) is carried out in accordance with known techniques. See, e.g., U.S. [Pat. Nos.] patents 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all [three] four U.S. [Patent] patents are incorporated herein by reference). In general, PCR involves, a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analysed to assess whether the sequence or sequences to be detected are present. Detection of the amplified sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophores, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al. Eds, Acad. Press, 1990).

Please substitute the following for the paragraph beginning on page 26, line 11 and ending on line 13

The functional derivatives of the present invention can be synthesized chemically or produced through recombinant DNA technology. [all]All these methods are well known in the art.

Please substitute the following for the paragraph beginning on page 28, line 24 and ending on page 29, line 4

As well, having identified ERRα as a target for lipogenesis, fatty acid esterification and fatty acid oxydation modulation, ERRα can be [be] used in a number of *in vitro* and *in vivo* assays to identify ligands therefor and dissect its structure/function relationship. Non limiting examples thereof include binding assays and the two hybrid system technology, as well known in the art (Ausubel et al., 1994, supra). This assay has proven beneficial to test compounds or a library

thereof. Thus, the invention also covers ERRα-expressing cells (prokaryotes, lower and higher eukaryotes) or variants thereof to identify mutations which modulate ERRα activity or [compunds] compounds which have ERRα modulating effects.

Please substitute the following for the paragraph beginning on page 29, line 5 and ending on line 20

The present invention also provides antisense nucleic acid molecules which can be used for example to decrease or abrogate the expression of the nucleic acid sequences or proteins of the present invention. An antisense nucleic acid molecule according to the present invention refers to a molecule capable of forming a stable duplex or triplex with a portion of its targeted nucleic acid sequence (DNA or RNA). The use of antisense nucleic acid molecules and the design and modification of such molecules is well known in the art as described for example in WO 96/32966, WO 96/11266, WO 94/15646, WO 93/08845 and [USP] U.S. patent 5,593,974. Antisense nucleic acid molecules according to the present invention can be derived from the nucleic acid sequences and modified in accordance to well known methods. For example, some antisense molecules can be designed to be more resistant to degradation to increase their affinity to their targeted sequence, to affect their transport to chosen cell types or cell compartments, and/or to enhance their lipid solubility [bu] by using nucleotide analogs and/or substituting chosen chemical fragments thereof, as commonly known in the art.

Please substitute the following for the paragraph beginning on page 34, line 17 and ending on page 35, line 6

[Figure 1 shows] Figs. 1A-1D show the targeted disruption of the *Estrra* gene and heterozygote inbreeding analysis. [*a*,] Fig. 1A, Structure of the ERRα locus, targeting vector, and recombinant allele. Top, map of the wild type locus: exons are indicated by black boxes. E2 encodes the upstream zinc-binding motif of the ERRα DNA-binding domain. Center, targeting construct. Bottom, map of the targeted allele, showing replacement of exon 2 sequences by the *neo*^{r} cassette. The restriction enzyme digests and the probes used to characterize the knockout mice are illustrated. B, *BamH*I; H, *Hind*III. [b,] Fig. 1B, Southern blot analysis of targeted ES clones. DNA from parental ES cells (R1) and two targeted clones (57 and 62) was digested with *BamH*I and hybridized to the 3' probe. The positions of bands corresponding to the wild-type

(10.7 kb) and targeted alleles (4.5 kb) are indicated (upper panel). Single integration of the targeting construct in targeted ES cell clones was confirmed with a *neo*^{r} probe: a single hybridizing band (6.0 kb) is present in the targeted lines (lower panel). [*c*,] Fig. 1C, Southern blot analysis of genotypes of 28d old pups from a heterozygote intercross: the litter contains viable homozygous null mice. [d,] Fig. 1D, Northern blot analysis of RNA obtained from the kidneys of the progeny of heterozygous intercrosses. ERRα expression is not detected in RNA samples obtained from homozygous null mutants.

Please substitute the following for the paragraph beginning on page 35, line 7 and ending on line 16

[Figure 2 shows] Figs. 2A-2D show the phenotypic analysis of *Estrra* null mutants. [a,] Fig. 2A, Mutant animals display decreased weight gain. Growth curves were performed by weighing animals at the indicated ages: both male and female knockout mice display significantly reduced body weight in comparison to their wild-type littermates. Arrows indicate start of pre-pubertal growth spurt. [*b*,] Fig. 2B, Body composition of *Estrra* null mice shows decreased ratio of fat to lean mass. [*c*,] Fig. 2C, *Estrra*$^{-/-}$ mice contain decreased body fat. Superficial carcass dissection of two 20 week old male mice shows the decreased body fat content of a 32.9 g knockout mouse (right) in comparison with his 38.1 g wild-type littermate (left). [*d*,] Fig. 2D, The difference in body composition is reflected by the relative sizes of the dissected fat pads.

Please substitute the following for the paragraph beginning on page 35, line 17 and ending on line 24

[Figure 3 shows] Fig. 3A-3C show the analysis of intestinal lipid transport in *Estrra* null mutants. [*a*,] Fig. 3A, Thin layer chromatographic analysis of tissue lipid content. The intestines of Estrra$^{-/-}$ mice contain decreased triglyceride and increased free fatty acids in comparison with their wild-type and heterozygous littermates. [*b*,] Fig. 3B, Analysis of glycerolipid synthesis in *Estrra* null mutants. Estrra[-/-] $^{-/-}$mice demonstrate reduced triglyceride synthesis in intestinal and hepatic whole cell extracts. [c,] Fig. 3C, Fat absorption profile. Estrra$^{-/-}$ mice and littermate controls display similar rates of absorption of radiolabeled oleic acid.

Please substitute the following for the paragraph beginning on page 35, line 25 and ending on page 36, line 2

[Figure 4 shows] Figs. 4A and 4B show the analysis of adipocyte function in Estrra null mutants. [a,] Fig. 4A, Histologic studies of epididymal fat pads show that *Estrra*$^{-/-}$ mice (lower panel) have decreased adipocyte volume in comparison to wild-type animals (upper panel). [*b*,] Fig. 4B, *Estrra*$^{-/-}$ mice demonstrate decreased lipogenesis in comparison to littermate controls.

Please substitute the following for the paragraph beginning on page 36, line 13 and ending on page 37, line 3

The method of production and the transgenic animals of the present invention are described herein below. In general, these animals are produced by engineering a nucleic acid construct which can disrupt the expression of the endogenous ERRα gene (i.e., the murine ERRα gene). Using known methods, this construct is amplified in bacterial cells, purified, and transferred into ES cells or isolated oocytes. The transfected ES cells can then be injected into blastocysts to generate chimeras. The chimeras which transmit the mutation to their offspring are identified and selected. These animals can then be used as founder animals to obtain different animal lines, derived from breeding with chosen animals. Heterozygous animals can then be produced and further mated to generate a hybrid F1 cross. Further matings of the F1 heterozygotes produce the wild type, heterozygous and homozygous null mutants of ERRα (having both copies of the ERRα gene disrupted). The homozygous animals can then serve in a number of experiments. Non-limiting examples thereof include[ ]: the characterization of their phenotype, and a reconstitution of the ERRα activity by complementation by a non-endogenous copy of a wild type ERRα gene or mutant or variant ERRα gene. An animal (or cells derived therefrom) expressing a mutant form of ERRα gene (from human, for example) could be used to screen for compounds which modulate more specifically the mutant form of the ERRα gene.

Please substitute the following for the paragraph beginning on page 37, line 4 and ending on line 11

The present invention therefore strongly indicates that ERRα is a direct regulator of fundamental cellular function. It is thus expected that this cellular function should occur [across] across species. The presence of the ERRα gene and its conservation among species (human, mice,

rats, fish and lower organisms; Escriva et al. (1997) Proc. Natl. Acad. Sci. USA 94:6803-6808), support its essential role in physiology. Thus, the antagonists identified by the methods and assays of the present invention should find a utility in the treatment of obesity and other metabolic diseases associated with ERRα malfunction in humans.

Please substitute the following for the paragraph beginning on page 37, line 12 and ending on line 13

The present invention is illustrated in further detail by the following non-[limitin] limiting examples.

Please substitute the following for the paragraph beginning on page 37, line 17 and ending on page 38, line 8

Three overlapping λ clones containing the mouse *Estrra* locus were isolated from a 129Sv genomic library (gift of Dr. A. Joyner, Skirball Institute, New York) and characterized by restriction mapping and direct sequencing of the exon boundaries. The knockout construct was created using pNT (Tybulewicz et al., 1991) and contained 6.4 [k]Kb of genomic DNA flanking the second exon of *Estrra*. An endfilled 4.2 Kb *BamHI/Not*I fragment, lying upstream of the second exon, was cloned into the *Xho*I site of pNT, while a 2.2 Kb *Hind*III fragment was cloned between the *neor* and TK cassettes to provide the 3' arm of the construct. Correct targeting of the *Estrra* locus replaces the receptor's second exon, which encodes a critical part of its DNA binding domain, with a *neo* cassette. The linearized construct was electroporated into R1 ES cells (Nagy and Rossant, 1993) which were selected with G418 (150 μg/mL) and gancyclovir (2 μM). Two ES cell clones were isolated and injected into C57BL6 blastocysts to generate chimeras, and three chimeras transmitted the mutation to their offspring. Heterozygous mice, generated by mating the chimeric animals with 129SvJ mice were mated with C57BL6 animals to generate hybrid F1 animals: physiologic studies were performed using the F2 null mutant and wild-type offspring obtained by mating the F1 hybrid heterozygotes. Complete disruption of the *Estrra* allele was verified by performing Northern blots using RNA obtained from placenta and kidneys of homozygous mutants.

Please substitute the following for the paragraph beginning on page 39, line 23 and ending on page 40. line 3

Mice were studied at 10:00h following free access to food overnight. The animals were conditioned by sham intraperitoneal injections of water. On the day of the experiment, the animals were injected intraperitoneally with ^3H_2O (0.5 mCi per 100 g body weight) and sacrificed by cervical dislocation 30 minutes later. Serum, adipose tissue and liver samples were harvested and stored at -80[o]^{o}C. The tissues were homogenized and heated in ethanolic KOH: the resulting extract, which contained saponified lipids, was acidified using concentrated sulfuric acid and extracted using petroleum ether. The extract was dried by evaporation and ^3H incorporation determined by scintillation counting.

Please substitute the following for the paragraph beginning on page 44, line 6 and ending on line 24

Previous studies have demonstrated that ERRα is highly expressed in brown adipose tissue (BAT) during murine development and that the receptor is upregulated during white and brown adipocyte differentiation *in vitro* (Sladek et al., 1997; Vega and Kelly, 1997). In addition, ERRα has been shown to modulate the activity of the medium chain acyl-coA dehydrogenase (MCAD) promoter, a key regulatory step in the fatty acid β-oxidation pathway (Sladek et al., 1997; Vega and Kelly, 1997). As dysregulation of BAT function has been associated with abnormalities of body composition, therefore, a characterization of BAT function in the ERRα knockout mice was carried out. ERRα null mutants had normal core body temperature and basal metabolic rate and displayed normal expression levels of uncoupling protein (UCP) mRNA in BAT (UCP-1) and skeletal muscle (UCP-2) (data not shown). Defects in fatty acid oxidation are frequently only apparent following situations of physiologic stress or food deprivation: neither prolonged cold exposure or fasts of up to 48 hour's duration resulted in any morbidity or mortality of Estrra[-/-]$^{-/-}$ mice (data not shown). Taken together, these data suggest that the abnormal body composition seen in ERRα null mutants was not a result of increased thermogenesis or increased basal energy expenditure, and that the animals did not have physiologically significant defects in fatty acid β-oxidation.

Please substitute the following for the paragraph beginning on page 44, line 25 and ending on page 45, line 15

Fat pads obtained from *Estrra* mutants displayed decreased adipocyte volume in comparison to wild-type animals (Fig. 4A), which suggests that the decreased adipose tissue mass observed in Estrra[-/-]$^{-/-}$ mice results from an imbalance between fatty acid synthesis and lipolysis rather than defects in adipocyte proliferation and differentiation. As ERRα expression is induced during early adipocyte differentiation *in vitro* (Sladek et al., 1997), it is possible that ERRα acts as a regulator of processes important for adipocyte function, such as fatty acid synthesis or esterification. In animals fed a standard laboratory diet, murine adipose tissue contains triglyceride formed from fatty acids that are synthesized *de novo* rather than from dietary lipid. Lipogenesis was assessed by treating *Estrra*$^{-/-}$ mice with $^{3}H_2O$: the amount of radioactive label incorporated into triacylglycerol can be measured by saponification and ether extraction of adipose tissues and other organs. *Estrra* null mutants demonstrate significantly decreased lipogenesis in comparison to littermate controls: in particular, knockout animals show a 30-55% decrease in ^{3}H incorporation into adipose tissue lipids and a 50% decrease in 3H incorporation into hepatic lipids (Fig. 4B). This observation demonstrates that adipose tissue of knockout mice possesses a defect in TG synthesis, which may result from decreased adipocyte and hepatic glycolysis activity, fatty acid synthesis or esterification.

Please substitute the following for the paragraph beginning on page 45, line 16 and ending on page 46, line 12

Experiments performed using the Estrra[-/-]$^{-/-}$ mice revealed that ERRα is a key regulator of fat metabolism, including intestinal fat transfer and esterification, as well as hepatic and adipocyte fat deposition. *Estrra*$^{-/-}$ mice display decreased fat content associated with reduced intestinal fatty acid esterification rates and abnormal regulation of fat deposition and mobilization in adipocytes and liver. Previous *in vitro* studies have demonstrated that ERRα modulates the expression of MCAD, a key regulatory enzyme of fatty acid β-oxidation, a pathway which may also play a role in establishing the ERRα phenotype. The relative importance of each of these effects in establishing the body composition of ERRα mice remains to be determined. Since the *Estrra*$^{-/-}$ mice show a normal level of energy intake, one would expect to observe an increase in energy expenditure to account for the decreased fat content of these mice. However, the sensitivity

of fecal fat measurements and calorimetry experiments may not be sufficient to identify small differences between wild-type and knockout animals which over a period of time would be sufficient to explain the observed phenotype. Within these experimental limitations, the data presented herein demonstrate that ERRα mice are lean as a result of aberrant regulation of peripheral lipid mobilization. ERRα mice display an unique combination of properties that suggests that modulation of ERRα activity may provide an effective method to regulate fat metabolism and that ERRα would be a key drug target for the treatment of obesity and other disorders of fat deposition. In addition, the close linkage of ESTRRA and diabetes susceptibility locus IDDM4 (Sladek et al., 1997) together with physiological defects observed in *Estrra*$^{-/-}$ mice suggests that drugs influencing ERRα activity could also be used to treat diabetes and other metabolic disorders.

In the Claims:

The following claims have been amended.

3. (Amended) The non-human transgenic animal of claim 1 [or 2], wherein said animal is a mammal.

5. (Amended) The non-human transgenic animal of claim[s] 1 [to 4], displaying a lean phenotype.

6. (Amended) The non-human transgenic animal of [one of] claim[s] 1 [to 5], whose germ cells and somatic cells additionally comprise a transgene encoding a non endogenous ERRα orphan nuclear receptor gene, wherein said transgene is expressed at levels sufficient to complement the disrupted endogenous ERRα orphan nuclear receptor activity.

9. (Amended) A cell line derived from the non-human transgenic animal of [one of] claim[s] 1 [to 8].

15. (Amended) A method for screening and identifying a compound which modulates ERRα orphan nuclear receptor activity, the method including:

a) exposing the non-human transgenic animal of [one of] claim[s] 5 [to 7] to a candidate compound, and;

b) determining the activity of said ERRα orphan nuclear receptor in said animal, wherein an increase in the receptor activity as compared to an unexposed non-human animal is indicative of a compound being capable of increasing ERRα orphan nuclear receptor activity, while a decrease in said receptor activity as compared to an unexposed non-human animal, is indicative of a compound being capable of decreasing ERRα orphan nuclear receptor activity.

18. (Amended) Method of identifying an agent which modulates fat and/or glucose metabolism *in vivo* comprising:

a) providing a promoter operably linked to a selectable or assayable marker, said promoter being modulated by ERRα;

b) measuring or selecting for said marker in a presence and in an absence of an agent suspected of modulating the promoter modulating activity of ERRα, thereby identifying an agent which modulates ERRα activity wherein a difference in the transcriptional activity in the presence of said agent, as compared to that in the absence thereof, identifies said agent as a modulator of ERRα activity;

c) administering said agent identified in b) to a non-human transgenic animal according to [one of] claim[s] 1 [to 7]; and

d) measuring lipid and/or glucose levels in said animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in lipid and/or glucose levels of the animal of step c) as compared to that of said control animal identifies said agent as a modulator of fat and/or glucose metabolism *in vivo*.

21. (Amended) The method of claim 20, wherein said mammal is a mouse [or human].

22. (Amended) A modulator of fat and/or glucose metabolism *in vivo* identified by [any one of] the method[s] of claim[s] 18[, 19, 20 or 21].

23. (Amended) A method of modulating fat tissue growth and/or weight gain, comprising:

a) administering to an animal an agent which modulates the promoter activity of a gene, wherein said promoter comprises cis-acting elements selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA;

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of i-iii)

such as to modulate the level of said gene, thereby modulating fat tissue growth and/or weight gain in said animal.

28. (Amended) A method of determining whether an agent modulates fat tissue growth and/or weight gain in an animal comprising:

a) providing a transcriptionally active preparation of ERRα or related factors and a DNA sequence comprising a promoter having a cis-acting sequence which modulates activity thereof by an interaction thereto of said ERRα and related factors;

b) measuring said transcriptional activity of said promoter or of a binding of at least ERRα or related factors to said cis-acting sequence in a presence and in an absence of an agent suspected of modulating the transcriptional activity of said promoter or the binding of said factors to said cis-acting sequence, thereby identifying an agent which modulates transcription of said promoter and wherein a difference in the transcriptional activity and/or binding in the presence of said agent, as compared to that in the absence thereof identifies said agent as a modulator of transcription;

c) administering said agent identified in b) to a non-human transgenic animal according to [one of] claim[s] 1 [to 7]; and

d) measuring fat tissue growth and/or weight gain in the animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in fat tissue growth and/or weight gain of the animal of step c) as compared to that of the control animal identifies said agent as a modulator of fat tissue growth and/or weight gain *in vivo*.

32. (Amended) A modulator of fat and/or glucose metabolism *in vivo* identified by [any one of] the method[s] of claim[s] 28[, 29, 30 or 31].

33. (Amended) A method of treating and/or preventing obesity, comprising administering to an obese animal, or an animal susceptible of becoming obese, an agent which modulates the promoter activity of a promoter comprising a cis-acting element selected from the group consisting of:

i) an estrogen response element;

ii) TGA AGG TCA;

iii) AGG TCA NNN TGA CCT (SEQ ID NO:1); and

iv) functional variants of [I]i-iii)

wherein cis-acting element is capable of binding to ERRα.

35. (Amended) A method of determining whether an agent modulates obesity in an animal comprising:

a) providing a transcriptionally active preparation of ERRα or related factors and a DNA sequence comprising a promoter having a cis-acting sequence which modulates activity thereof by an interaction thereto of said ERRα and related factors;

b) measuring said transcriptional activity of said promoter or of a binding of at least ERRα or related factors to said cis-acting sequence in a presence and in an absence of an agent suspected of modulating the transcriptional activity of said promoter or the binding of said factors to said cis-acting sequence, thereby identifying an agent which modulates transcription of said promoter and wherein a difference in the transcriptional activity and/or binding in the presence of said agent, as compared to that in the absence thereof identifies said agent as a modulator of transcription;

c) administering said agent identified in b) to a non-human transgenic animal according to [one of] claim[s] 1 [to 7]; and

d) assessing obesity in the animal of step c) and comparing same with that of a control animal, not having been administered said agent, wherein a difference in obesity of the

animal of step c) as compared to that of the control animal identifies said agent as a modulator of obesity *in vivo*.

38. (Amended) The method of claim 37, wherein said mammal is a mouse [or human].

39. (Amended) A modulator of glucose or fat metabolism *in vivo* identified by [any one of] the method[s] of claim[s] 35[, 36, 37 or 38].